more accurately study plant-*P. cinnamomi* interactions in plants, and to be particularly useful in naturally infected material.

Histological changes of *Quercus ilex* seedlings infected by *Phytophthora cinnamomi*

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Since the early 1980s, severe decline and occasional sudden death of extensive areas of holm oak (*Quercus ilex*) and cork oak (*Q. suber*) woodlands in the southwest of the Iberian Peninsula have been associated to the damage caused by *Phytophthora cinnamomi*, an alien and invasive pathogen infecting roots of many woody species. The objective of this work was to perform histological studies of holm oak infected by *P. cinnamomi*. To achieve this, *Q. ilex* subsp*. ballota* acorns were collected in Extremadura (Spain) and germinated in sterile vermiculite. Five-week-old seedlings were inoculated by immersing them in flasks with *P. cinnamomi* colonised agar and soil extract solution. Root sections were obtained every 12 hours, during 7 days. Root tissue was fixed with FAA and Karnovsky fixative and processed afterwards using different microscopic techniques. Low temperature scanning electron microscopy (LTSEM) showed hyphae and encysted zoospores 24 h after inoculation. Root sections (10-15 µm) treated with calcofluor white were observed under epifluorescence microscopy and *P. cinnamomi* hyphae covering root surface and going progressively through cortex tissue were also observed. Roots sections (8-10 m) stained with safranin-fast green and observed under light microscopy showed hyphae on the external root tissue 24 h after inoculation. Light microscopy was also used with 2 µm root sections stained with toluidine blue and allowed the detection of *P. cinnamomi* hyphae penetrating the parenchyma tissue. Finally, 80 nm root slices were obtained and examined through transmission electron microscopy (TEM). *P. cinnamomi* penetrated the cell walls forming haustorial-like structures and also grew through intercellular spaces.

Quick dissemination of *Phytophthora cinnamomi* threatens biodiversity in a World Heritage Site (Doñana Biological Reserve, SW Spain)

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Scattered big trees play a main role in maintaining biodiversity in savannah-like ecosytems. In this study we analyze the case of a remnant centenarian Cork oak (*Quercus suber* L.) population in the Doñana Biological Reserve (DBR, SW Spain), a Biosphere Reserve. Previous studies demonstrated that it was threatened by herbivorous and nesting wading birds proliferation [1]. Several sudden dieback events not related to any of previously reported causes lead us to investigate the potential role of pathogenic oomycetes.

Along four years of study period (2008-2011) tree rizosphere was increasingly being colonized by *Phytophthora cinnamomi.* We found that 2010 late winter/ early spring rainfall values exceeded all previous (32 yr) records and significantly extended the period with flooding/high soil moisture towards warmer months. These outstanding climatic conditions seem to have favoured the massive spread of this invasive water-dependent pathogen.

On the other hand, we found a significant correlation between the occurrence of *P. cinnamomi* in 2008/9 and tree crown health status in late 2010, suggesting a delay between pathogen arrival and the appearance of crown symptoms.

We analyzed future perspectives for pathogen spreading and discussed the feasibility of implementing currently available control measures in the context of a highly protected biodiversity reserve. We conclude that only individual treatments could allow for simultaneously save infected trees, prevent infections on healthy trees and avoid any chemical release to the environment.

[1] García, L.V., Ramo, C., Aponte, C. Moreno, A., Dominguez, M. T., Gómez-Aparicio, L., Redondo, R and Marañón T. Protected wading bird species threaten relict centennial cork oaks in a Mediterranean Biosphere Reserve: a conservation management conflict . *Biological Conservation* **144** 764–771 (2011).